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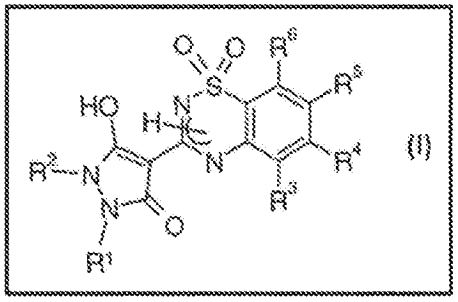
Abstract: Compounds useful as HCV anti-infectives having the formula (I) wherein the formula variables are as defined herein, are disclosed. Also disclosed are methods of making and using the same.



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ANTI-INPECTIVES FIELD OF THE INVENTION The present invention relates to compounds that inhibit an RNA-containing virus and methods of using the same. Specifically, the present invention relates to hepatitis C virus (HCV) inhibitors and methods of using the same.

BACKGROUND OF THE INVENTION In the U. S. , an estimated 4.5 million Americans are chronically infected with HCV.

Although only 30% of acute infections are symptomatic, greater than 85% of infected individuals develop chronic, persistent infection. Treatment costs for HCV infection have < been estimated at $5.46 billion for the U. S. in 1997. World-wide, over 200 million people are estimated to be infected chronically. HCV infection is responsible for 40-60% of all chronic liver disease and 30% of all liver transplants. The CDC estimates that the number of deaths due to HCV will minimally increase to 38,000/yr. by the year 2010.

Due to the high degree of variability in the viral surface antigens, existence of multiple viral genotypes, and demonstrated specificity of immunity, the development of a successful vaccine in the near future is unlikely. Alpha-interferon (alone or in combination with ribavirin) has been widely used since its approval for treatment of chronic HCV infection. However, adverse side effects are commonly associated with this treatment: flu- like symptoms, leukopenia, thrombocytopenia, and depression from interferon, as well as hemolytic anemia induced by ribavirin (Lindsay, K. L. (1997) Hepatology 26 (suppl 1): 71S- 77S). This therapy remains less effective against infections caused by HCV genotype 1 (which constitutes-75% of all HCV infections in the developed markets) compared to infections caused by the other 5 major HCV genotypes. Unfortunately, only-50-80% of the patients respond to this treatment (measured by a reduction in serum HCV RNA levels and normalization of liver enzymes) and, of those treated, 50-70% relapse within 6 months of cessation of treatment. Recently with the introduction of pegylated interferon (Peg-IFN), both initial and sustained response rates have improved substantially, and combination treatment of Peg-IFN with ribavirin constitutes the gold standard for therapy. However, the side effects associated with combination therapy and the impaired response in patients with genotype 1 present opportunities for improvement in the management of this disease.

First identified by molecular cloning in 1989 (Choo, Q-L. et al., (1989) Science 244: 359-362), HCV is now widely accepted as the most common causative agent of post- transfusion non A, non-B hepatitis (NANBH) (Kuo, G. et al., (1989) Science 244: 362-364).

Due to its genome structure and sequence homology, this virus was assigned as a new genus in the Flaviviridae family. Like the other members of the Flaviviridae (such as flaviviruses (e. g. , yellow fever virus and Dengue virus types 1-4) and pestiviruses (e. g. , bovine viral diarrhea virus, border disease virus, and classic swine fever virus (Choo et al., 1989; Miller, R. H. and R. H. Purcell (1990) Proc. Natl. Acad. Sci. USA 87: 2057-2061)), HCV is an enveloped virus containing a single strand RNA molecule of positive polarity. The HCV genome is approximately 9.6 kilobases (kb) with a long, highly conserved, noncapped 5' nontranslated region (NTR) of approximately 340 bases which functions as an internal ribosome entry site (IRES) (Wang CY. Le SY. Ali N. Siddiqui A. , Rna-A Publication of the Rna Society. 1 (5): 526-537,1995 Jul). This element is followed by a region which encodes a single long open reading frame (ORF) encoding a polypeptide of-3000 amino acids comprising both the structural and nonstructural viral proteins.

Upon entry into the cytoplasm of the cell, the HCV-RNA is directly translated into a polypeptide of-3000 amino acids comprising both the structural and nonstructural viral proteins. This large polypeptide is subsequently processed into the individual structural and nonstructural proteins by a combination of host and virally-encoded proteinases (Rice, C. M.

(1996) in B. N. Fields, D. M. Knipe and P. M. Howley (Eds.) Virology, 2nd Edition, p931- 960, Raven Press, NY). Following the termination codon at the end of the long ORF, there is a 3'NTR which roughly consists of three regions: an-40 base region which is poorly conserved among various genotypes, a variable length poly (U) /polypyrimidine tract, and a highly conserved 98 base element also called the"3'X-tail" (Kolykhalov, A. et al., (1996) J.

Virology 70: 3363-3371 ; Tanaka, T. et al., (1995) Biochem Biophys. Res. Commun.

215: 744-749; Tanaka, T. et al., (1996) J. Virology 70: 3307-3312; Yamada, N. et al., (1996) Virology 223: 255-261). The 3'NTR is predicted to form a stable secondary structure that is essential for HCV growth in chimps and is believed to function in the initiation and regulation of viral RNA replication.

The NS5B protein (591 amino acids, 65 kDa) of HCV (Behrens, S. E., et al., (1996) EMBO J. 15: 12-22), encodes an RNA-dependent RNA polymerase (RdRp) activity and contains canonical motifs present in other RNA viral polymerases. The NS5B protein is fairly well conserved both intra-typically (-95-98% amino acid (aa) identity across 1b isolates) and inter-typically (-85% aa identity between genotype la and lb isolates). The essentiality of the HCV NS5B RdRp activity for the generation of infectious progeny virions has been formally proven in chimpanzees (Kolykhalov, A. A., et al., (2000) J.

Virology 74: 2046-2051). Thus, inhibition of NS5B RdRp activity (inhibition of RNA replication) is predicted to cure HCV infection.

Positive strand hepatitis C viral RNA is the nucleic acid strand which is translated and initially copied upon entry of the HCV-RNA into the cell. Once in the cell, positive strand viral RNA generates a negative strand replicative intermediate. Negative strand RNA is the template used to generate the positive strand message which is generally packaged into productive virions. Presently, HCV inhibitor compounds are only evaluated for their ability to inhibit positive strand HCV-RNA. However, it would be desirable to develop inhibitor compounds having the ability to inhibit both positive and negative strand replication to obtain complete clearance of the HCV virus.

Accordingly, there exists a significant need to identify synthetic or biological compounds for their ability to inhibit HCV. Preferably, such synthetic or biological compounds inhibit both positive and negative strand replication of the hepatitis C virus.

SUMMARY OF THE INVENTION This invention is directed to a compound having Formula I, as follows: wherein: R'and R2 are each independently selected from the group consisting of: C1-C10 alkyl, C2-C10 alkenyl, C2-Clo alkynyl, C3-C8 cycloalkyl, aryl or heteroaryl, where said alkyl, alkenyl, alkynyl, cycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from Cl-C6 alkyl, C3-C6 cycloalkyl, heterocycloalkyl, aryl, heteroaryl, halogen, nitro, cyano,-C (O) OR7,-C (O) R7,-OR7,-SR', -S(O)R7, -S(O2)R7, -SO2NR8R9, -CONR8R9, -N(R8) C (O) R7,-N (R8) C (O) OR7, -OC (O) NR8R9,-N (R8) C (O) NR8R9,-P (O) (OR7) 2,-SO2NR$R9,-N (R8) SO2R7, and-NR8R9 ; wherein said cycloalkyl, heterocycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from-NRR', Cl-C6 alkyl, Cl-C6 haloalkyl, halogen, cyano and nitro; R3 is hydrogen, halogen, cyano, Cl-C6 alkyl,-OH, or-OC-C4 alkyl ; R4 is hydrogen, halogen, cyano, Cl-C6 alkyl,-OH,-OCl-C4 alkyl, Cl-C4 haloalkyl, nitro or amino; Rs is H, nitro, cyano, halogen,-C (O) OR',-C (O) R7,-OR7,-SR7,-S (O) R7,-S (O) 2R7, -NR8R9, protected -OH, -CONR8R9, -N(R8) C (O) R7,-N (R8) C (O) OR7, -OC (O) NR8R9, -N(R8) C (O) NR8R9,-P (O) (OR7) 2, -SO2NR8R9, -N (R8) SO2R7, Cl-Clo alkyl, C2-Clo alkenyl, Cl-calo alkynyl, C3-C6 cycloalkyl, aryl or heteroaryl, where said alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is unsubstituted or substituted with one or more substituents independently selected from Cl-C6 alkyl, Cl-C6 haloalkyl, aryl, heteroaryl, halogen,-OR7, -SR7,-NR8R9, cyano and nitro; R6 is H, halogen, Cl-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, -OR7, -SR7, -NR8R9, cyano or nitro; wherein each R7 is independently selected from the group consisting of H, Cl-Cl0 alkyl, C2-Clo alkenyl, C2-Clo alkynyl, C3-C8 cycloalkyl, heterocycloalkyl, C6-C12 aryl, heteroaryl, or C6-Cl2 aryl-Cl C6 alkyl-, where said alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl or arylalkyl group is unsubstituted or substituted with one or more substituents independently selected from Cl-C4 alkyl, haloalkyl, halogen, hydroxyl,-O (Cl-C4 alkyl), cyano, nitro,-N (C1-C4 alkyl) (C1-C4 alkyl), -NH(C1-C4 alkyl), -NH2, aryl, heteroaryl,-CO2 (Cl-C4 alkyl), -CO2H, -CON(C1-C4 alkyl)(C1-C4 alkyl), -CONH (C1-C4 alkyl), -CONH2, -N(C1-C4 alkyl) CON (C1-C4 alkyl) (C1-C4 alkyl),-NHCON (C1-C4 alkyl) (C1-C4 alkyl), -NHCONH(C1-C4 alkyl),-OCON(C1-C4 alkyl)(C1-C4 alkyl), -CONH(C1-C4 alkyl), OCONH2,-SO2N(C1-C4 alkyl) (C1-C4 alkyl),-SO2NH (Cl-C4 alkyl),-CO (Cl-C4 alkyl), - CO (aryl) and-CO (heteroaryl); and R8 and R9 are each independently selected from H, Cl-Cl0 alkyl, C3-C8 cycloalkyl, heterocycloalkyl, Ce-ci aryl, heteroaryl, C3-C8 cycloalkyl-Cl-C6 alkyl-, heterocycloalkyl-Cl-C6 alkyl-, C6-C12 aryl-C1-C6 alkyl-or heteroaryl-Cl-C6 alkyl-, or R8 and R9 taken together with the nitrogen to which they are attached represent a 5-or 6-membered saturated ring optionally containing one other heteroatom selected from oxygen, sulfur, and nitrogen, where said alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkylalkyl, arylalkyl, heteroarylalkyl or 5-or 6-membered ring is unsubstituted or substituted with one or more substituents independently selected from C1-C4 alkyl, Cl-C4 haloalkyl, halogen, hydroxyl,-O (C1-C4 alkyl),

cyano, nitro, -N(C1-C4 alkyl)(C1-C4 alkyl), -NH(C1-C4 alkyl), -NH2, aryl, heteroaryl, -CO2(C1-C4 alkyl), -CO2H, -CON (C1-C4 alkyl) (C1-C4 alkyl),-CONH (Cl-C4 alkyl),-CONH2, (C1-C4 alkyl) CON (Ci-C4 alkyl)(C1-C4 alkyl), -NHCON(C1-C4 alkyl)(C1-C4 alkyl), -NHCONH(C1-C4 alkyl), -OCON (Cl-C4 alkyl) (Cl-C4 alkyl), -OCONH (Cl-C4 alkyl),-SO2N (C-C4 alkyl) (Cl-C4 alkyl),-SO2NH (Cl-C4 alkyl), -CO (Cl-C4 alkyl),-CO (aryl) and-CO (heteroaryl); or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

This invention is also directed to a prodrug of a compound according to Formula I, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof. In addition, this invention is directed to pharmaceutical compositions comprising a compound according to Formula I, or a tautomer thereof, or a prodrug thereof, or salts or solvates thereof.

In another embodiment, this invention is directed to a method of inhibiting an RNA- containing virus comprising contacting the virus with an effective amount of a compound of Formula I. In yet another embodiment, this invention is directed to a method of treating infection or disease caused by an RNA-containing virus which comprises administering to a subject in need thereof, an effective amount of a compound according to Formula I. This invention is particularly directed to methods of inhibiting hepatitis C virus. This invention is also directed to a method for inhibiting replication of hepatitis C virus which comprises inhibiting replication of both positive and negative strand HCV-RNA.

DETAILED DESCRIPTION OF THE INVENTION It will be appreciated by those skilled in the art that the compounds of this invention, represented by generic Formula I, above, exist in tautomeric forms having Formula I-A and Formula I-B, as follows: I-A I-B In addition, it will be appreciated by those skilled in the art, that the compounds of this invention may exist in several other tautomeric forms. All tautomeric forms of the compounds described herein are intended to be encompassed within the scope of the present invention. Examples of some of the other possible tautomeric forms of the compounds of this invention include, but are not limited to: I-C I-D I-E As a convention, the compounds exemplified herein have been assigned names based on the structure of the tautomer of Formula I-A. It is to be understood that any reference to such named compounds is intended to encompass all tautomers of the named compounds and any mixtures of tautomers of the named compounds.

As used herein, the term"alkyl"represents a straight-or branched-chain saturated hydrocarbon which may be unsubstituted or substituted by one or more of the substituents defined herein. Exemplary alkyls include, but are not limited to methyl (Me), ethyl (Et), propyl, isopropyl, butyl, isobutyl, t-butyl and pentyl. The term"lower alkyl"refers to an alkyl containing from 1 to 4 carbon atoms.

When the term"alkyl" (or alkenyl or alkynyl) is used in combination with other substituent groups, such as"haloalkyl"or"arylalkyl", the term"alkyl"is intended to encompass a divalent straight or branched-chain hydrocarbon radical. For example, "cycloalkylalkyl"is intended to mean the radical-alkyl-cycloalkyl, wherein the alkyl moiety thereof is a divalent straight or branched-chain hydrocarbon radical and the cycloalkyl moiety thereof is as defined as above, and is represented by the bonding arrangement present in the groups-CH2-cyclopropyl,-CH2-cyclohexyl, or -CH2 (CH3) CHCH2-cyclopentenyl."Arylalkyl"is intended to mean the radical-alkylaryl, wherein the alkyl moiety thereof is a divalent straight or branched-chain carbon radical and the aryl moiety thereof is as defined as above, and is represented by the bonding arrangement present in a benzyl group (-CH2-phenyl).

As used herein, the term"alkenyl"represents a straight-or branched-chain hydrocarbon containing one or more carbon-carbon double bonds. An alkenyl may be unsubstituted or substituted by one or more of the substituents defined herein. Exemplary alkenyls include, but are not limited ethenyl, propenyl, butenyl, isobutenyl and pentenyl.

As used herein, the term"alkynyl"represents a straight-or branched-chain hydrocarbon containing one or more carbon-carbon triple bonds and, optionally, one or more carbon-carbon double bonds. An alkynyl may be unsubstituted or substituted by one or more of the substituents defined herein. Exemplary alkynyls include, but are not limited ethynyl, butynyl, propynyl (propargyl, isopropynyl), pentynyl and hexynyl.

"Cycloalkyl"represents a group comprising a non-aromatic monocyclic, bicyclic, or tricyclic hydrocarbon containing from 3 to 14 carbon atoms which may be unsubstituted or substituted by one or more of the substituents defined herein and may be saturated or partially unsaturated. Exemplary cycloalkyls include monocyclic rings having from 3-7, preferably 3-6, carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl and

cycloheptyl.

"Heterocycloalkyl"represents a group comprising a non-aromatic, monovalent monocyclic, bicyclic, or tricyclic radical, which is saturated or partially unsaturated, containing 3 to 18 ring atoms, which includes 1 to 5 heteroatoms selected from nitrogen, oxygen and sulfur, and which may be unsubstituted by one or more of the substituents defined herein, and to which may be fused one or more cycloalkyl rings, which may be unsubstituted or substituted by one or more substituents defined herein. Illustrative examples of heterocycloalkyl groups include, but are not limited to, azetidinyl, pyrrolidyl, piperidyl, piperazinyl, morpholinyl, tetrahydro-2H-1, 4-thiazinyl, tetrahydrofuryl, dihydrofuryl, tetrahydrothienyl, dihydrothienyl, oxazolinyl, thiazolinyl, pyrazolinyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, dihydrothiopyranyl, 1,3- dioxolanyl, 1,3-dioxanyl, 1,4-dioxanyl, 1,3-oxathiolanyl, 1,3-oxathianyl, 1,3-dithianyl, azabicylo [3.2. 1] octyl, azabicylo [3.3. 1] nonyl, azabicylo [4.3. 0] nonyl, oxabicylo [2.2. 1] heptyl and 1,5, 9-triazacyclododecyl. Generally, in the compounds of this invention, the heterocycloalkyl group is a monocyclic heterocycloalkyl, such as azetidinyl, pyrrolidyl, piperidyl, piperazinyl, morpholinyl, tetrahydro-2H-1, 4-thiazinyl, tetrahydrofuryl, tetrahydrothienyl, dihydrofuryl, tetrahydropyranyl, dihydropyranyl, 1,3-dioxolanyl, 1,3- dioxanyl, 1,4-dioxanyl, 1,3-oxathianyl, 1,3-dithianyl, oxazolinyl, thiazolinyl and pyrazolinyl.

"Aryl"represents a group comprising an aromatic, monovalent monocyclic, bicyclic, or tricyclic radical containing from 6 to 18 carbon ring atoms, which may be unsubstituted or substituted by one or more of the substituents defined herein, and to which may be fused one or more cycloalkyl rings, which may be unsubstituted or substituted by one or more substituents defined herein. Generally, in the compounds of this invention, aryl is phenyl.

"Heteroaryl"represents a group comprising an aromatic monovalent monocyclic, bicyclic, or tricyclic radical, containing 5 to 18 ring atoms, including 1 to 5 heteroatoms selected from nitrogen, oxygen and sulfur, which may be unsubstituted or substituted by one or more of the substituents defined herein, and to which may be fused one or more cycloalkyl rings, heterocycloalkyl rings or aryl rings, which may be unsubstituted or substituted by one or more substituents defined herein. This term also encompasses bicyclic or tricyclic heterocyclic-aryl compounds containing an aryl ring moiety fused to a heterocycloalkyl ring moiety, containing 5 to 16 ring atoms, including 1 to 5 heteroatoms selected from nitrogen, oxygen and sulfur, which may be unsubstituted or substituted by one or more of the substituents defined herein. Illustrative examples of heteroaryl groups include, but are not limited to, thienyl, pyrrolyl, imidazolyl, pyrazolyl, furyl, isothiazolyl, furazanyl, oxazolyl, oxadiazolyl, isoxazolyl, thiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazinyl, triazolyl, tetrazolyl, benzo [b] thienyl, naphtho [2,3- b] thianthrenyl, [1, 2,3] thiadiazolyl, isobenzofuryl, chromenyl, xanthenyl, phenoxathienyl, indolizinyl, isoindolyl, indolyl, indazolyl, purinyl, isoquinolyl, quinolyl, phthalazinyl, naphthridinyl, quinzolinyl, benzothiazolyl, benzimidazolyl, tetrahydroquinolinyl, cinnolinyl, pteridinyl, carbozolyl, beta-carbolinyl, phenanthridinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, isothiazolyl, phenathiazinyl, and phenoxazinyl. Generally, in the compounds of this invention, the heteroaryl group is a monocyclic heteroaryl, such as thienyl, pyrrolyl, imidazolyl, pyrazolyl, furyl, isothiazolyl, furazanyl, isoxazolyl, oxazolyl, oxadiazolyl, thiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazinyl, triazolyl, and [1, 2,3] thiadiazolyl.

The terms"halogen"and"halo"represent chloro, fluoro, bromo or iodo substituents.

"Hydroxyl"is intended to mean the radical-OH. "Alkoxy"is intended to mean the radical - OR, where Ra is an optionally substituted alkyl group. Exemplary alkoxy include methoxy, ethoxy, propoxy, and the like. "Lower alkoxy"groups have optionally substituted alkyl moieties from 1 to 4 carbons. "Alkylenedioxy"is intended to mean the divalent radical - which is bonded to adjacent atoms (e. g. , adjacent atoms on a phenyl or naphthyl ring), wherein Ra is a Cl-C2 alkyl group. Exemplary alkylenedioxy-substituted phenyls include benzo [1, 3] dioxyl and 2,3-dihydro-benzo [1, 4] dioxyl.

Another embodiment of this invention comprises a compound of Formula I, wherein: R'is C3-C8 alkyl, C3-C6 cycloalkyl-Cl-C4 alkyl-, C6 aryl-Cl-C4 alkyl-, monocyclic heterocycloalkyl-Cl-C4 alkyl-or monocyclic heteroaryl-Cl-C4 alkyl-. In specific embodiments of this invention, Rl is iso-pentyl, benzyl, or 3,3-dimethylpentyl.

In another embodiment of this invention, R2 is Cl-C6 alkyl, C3-C6 cycloalkyl, monocyclic heterocycloalkyl, phenyl, monocyclic heteroaryl, C3-C6 cycloalkyl-Cl-C4 alkyl-, C6 aryl-Cl-C4 alkyl-, monocyclic heterocycloalkyl-Cl-C4 alkyl-or monocyclic heteroaryl-Cl-C4 alkyl-. In specific embodiments, R2 is tert-butyl.

In another embodiment of this invention, R3, R4 and R6 are each H.

In yet another embodiment of this invention, Rs is H, Cl-C4 alkyl,-OH or - O (Cl-C4 alkyl), wherein said Cl-C4 alkyl or-O (Cl-C4 alkyl) is unsubstituted or substituted by-NH2,-CN,-CONH2,-CON (Cl-C4 alkyl) (Cl-C4 alkyl),-CONH (Ra), aryl or heteroaryl, wherein the aryl or heteroaryl is optionally unsubstituted or substituted by one or more substituents selected from the group consisting of cyano, halogen, -C (O) O (Ci-C6 alkyl), -C (O) C,-C6 alkyl,-ORa,-NRaRa,-CONRaRa, and-OCONRaRa, wherein each Ra is independently H or C-C4 alkyl. In specific embodiments of this invention, Rs is H.

A preferred embodiment of this invention comprises compounds of Formula I wherein: RI is C3-C6 alkyl or benzyl; R2 is C3-C6 alkyl ; R3, R4, R5, R6 are each H; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

It is to be understood that the present invention encompasses all combinations of particular, specific and preferred groups described hereinabove.

If a substituent described herein is not compatible with the synthetic methods of this invention, the substituent may be protected with a suitable protecting group that is stable to the reaction conditions used in these methods. The protecting group may be removed at a suitable point in the reaction sequence of the method to provide a desired intermediate or target compound. Suitable protecting groups and the methods for protecting and de- protecting different substituents using such suitable protecting groups are well known to those skilled in the art; examples of which may be found in T. Greene and P. Wuts, Protecting Groups in Cllemical Syntl1esis (3rd ed.), John Wiley & Sons, NY (1999), which is incorporated herein by reference in its entirety. In some instances, a substituent may be specifically selected to be reactive under the reaction conditions used in the methods of this invention. Under these circumstances, the reaction conditions convert the selected substituent into another substituent that is either useful as an intermediate compound in the methods of this invention or is a desired substituent in a target compound.

In the compounds of this invention, various substituents may be a"protected-OH" group. This term refers to a substituent represented as-ORP, where RP refers to a suitable protecting group for an-OH moiety. Hydroxyl protecting groups are well known in the art and any hydroxyl protecting group that is useful in the methods of preparing the compounds of this invention may be used. Exemplary hydroxyl protecting groups include benzyl, tetrahydropyranyl, silyl (trialkyl-silyl, diaryl-alkyl-silyl, etc.) and various carbonyl-containing protecting groups, as disclosed in T. Greene and P. Wuts, supra.

The compounds of this invention may contain at least one chiral center and may exist as single stereoisomers (e. g. , single enantiomers), mixtures of stereoisomers (e. g. any mixture or enantiomers or diastereomers) or racemic mixtures thereof. All such single stereoisomers, mixtures and racemates are intended to be encompassed within the broad scope of the present invention. Compounds identified herein as single stereoisomers are meant to describe compounds that are present in a form that are at least 90% enantiomerically pure. Where the stereochemistry of the chiral carbons present in the chemical structures illustrated herein is not specified, the chemical structure is intended to encompass compounds containing either stereoisomer of each chiral center present in the compound. Such compounds may be obtained synthetically, according to the procedures described herein using optically pure (enantiomerically pure) or substantially optically pure materials. Alternatively, these compounds may be obtained by resolution/separation of a mixture of stereoisomers, including racemic mixtures, using conventional procedures.

Exemplary methods that may be useful for the resolution/separation of mixtures of stereoisomers include chromatography and crystallization/re-crystallization. Other useful methods may be found in"Efzantiomers, Racemates, and Resolutions,"J. Jacques et al., 1981, John Wiley and Sons, New York, NY, the disclosure of which is incorporated herein by reference.

The compounds of this invention may possess one or more unsaturated carbon- carbon double bonds. All double bond isomers, both the cis (Z) and trans (E) isomers, and mixtures thereof are intended to be encompassed within the scope of the present invention.

The term"pharmaceutically acceptable salt"is intended to describe a salt that retains the biological effectiveness of the free acid or base of a specified compound and is not biologically or otherwise undesirable.

If an inventive compound is a base, a desired salt may be prepared by any suitable method known in the art, including

treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, or with an organic acid, such as acetic acid, trifluoroacetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acid, such as glucuronic acid or galacturonic acid, alpha-hydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid or the like. Additional examples of pharmaceutically acceptable salts include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates succinates, suberates, sebacates, fumarates, maleates, butyne-1, 4-dioates, hexyne-1, 6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, phenylacetates, phenylpropionates, phenylbutrates, citrates, lactates, y-hydroxybutyrates, glycollates, tartrates mandelates, and sulfonates, such as xylenesulfonates, methanesulfonates, propanesulfonates, naphthalene-1-sulfonates and naphthalene-2-sulfonates.

If an inventive compound is an acid, a desired salt may be prepared by any suitable method known to the art, including treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary, or tertiary); an alkali metal or alkaline earth metal hydroxide; or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine; ammonia; primary, secondary, and tertiary amines; and cyclic amines, such as ethylene diamine, dicyclohexylamine, ethanolamine, piperidine, morpholine, and piperazine; as well as inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum, and lithium.

Because the compounds of this invention may contain both acid and base moieties, pharmaceutically acceptable salts may be prepared by treating these compounds with an alkaline reagent or an acid reagent, respectively. Accordingly, this invention also provides for the conversion of one pharmaceutically acceptable salt of a compound of this invention, e. g. , a hydrochloride salt, into another pharmaceutically acceptable salt of a compound of this invention, e. g. , a mesylate salt or a sodium salt.

The term"solvate"is intended to mean a pharmaceutically acceptable solvate form of a specified compound that retains the biological effectiveness of such compound.

Examples of solvates include compounds of the invention in combination with water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine. In the case of compounds, salts, or solvates that are solids, it is understood by those skilled in the art that the inventive compounds, salts, or solvates may exist in different crystal forms, all of which are intended to be within the scope of the present invention and specified formulas.

Also included within the scope of this invention are prodrugs of the compounds of this invention. The term"prodrug"is intended to mean a compound that is converted under physiological conditions, e. g. , by solvolysis or metabolically, to a compound of Formula I, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof. A prodrug may be a derivative of one of the compounds of this invention that contains, for example, a carboxylic acid ester or amide moiety or an enol-ester moiety that may be cleaved under physiological conditions. A prodrug containing such a moiety may be prepared according to conventional procedures, for example, by treatment of a compound of Formula I, containing an amino, amido or hydroxyl moiety with a suitable derivatizing agent, for example, a carboxylic acid halide or acid anhydride, or by converting a compound of Formula I, containing a carboxyl moiety to an ester or amide or by converting a compound of Formula I, containing a carboxylic acid ester moiety to an enol-ester. Prodrugs of the compounds of this invention may be determined using techniques known in the art, for example, through metabolic studies. See, e. g. ,"Design of Prodrugs, " (H. Bundgaard, Ed.) 1985, Elsevier Publishers B. V. , Amsterdam, The Netherlands.

The present invention is also directed to a method of inhibiting an RNA-containing virus which comprises contacting the virus with an effective amount of a compound of Formula I. This invention is also directed to a method of treating infection or disease caused by an RNA-containing virus comprising administering to a subject in need thereof, an effective amount of the compound of Formula I. Specifically, this invention is directed to a method of inhibiting HCV activity, comprising contacting the virus with an effective amount of a compound of Formula I, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof. For example, HCV activity may be inhibited in mammalian tissue by administering a compound of Formula I or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

A therapeutically"effective amount"is intended to mean that amount of a compound that, when administered to a mammal in need of such treatment, is sufficient to effect treatment, as defined herein. Thus, e. g. , a therapeutically effective amount of a compound of Formula I or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof is a quantity of an inventive agent that, when administered to a mammal in need thereof, is sufficient to modulate or inhibit the activity of HCV such that a disease condition which is mediated by that activity is reduced or alleviated. The amount of a given compound that will correspond to such an amount will vary depending upon factors such as the particular compound (e. g. , the potency (iso), efficacy (ECso), and the biological half-life of the particular compound), disease condition and its severity, the identity (e. g. , age, size and weight) of the mammal in need of treatment, but, nevertheless, can be routinely determined by one skilled in the art. Likewise, the duration of treatment and the time period of administration (time period between dosages and the timing of the dosages, e. g. , before/with/after meals) of the compound will vary according to the identity of the mammal in need of treatment (e. g. , weight), the particular compound and its properties (e. g., pharmaceutical characteristics), disease or condition and its severity and the specific composition and method being used, but, nevertheless, can be routinely determined by one of skill in the art.

In addition, this invention is directed to a method for inhibiting replication of hepatitis C virus comprising inhibiting replication of both positive and negative strand HCV-RNA, which method comprises contacting a cell infected with said virus with an effective amount of a compound of Formula I. This invention is also directed to a method of treating infection or disease caused by hepatitis C virus comprising inhibiting replication of both positive and negative strand HCV-RNA, which method comprises administering to a subject in need thereof, an effective amount of a compound of Formula I. More specifically, this invention is directed to a method of inhibiting replication of both positive and negative strand HCV-RNA with a compound of Formula I, wherein the compounds demonstrate substantially equal inhibition of positive strand HCV-RNA replication and negative strand HCV-RNA replication. That is, for a given compound of this invention, the ICso for inhibition of positive strand HCV-RNA replication is not statistically different (less than a 2-fold difference) from the ICso for inhibition of negative strand HCV-RNA replication.

Generally, the compounds of this invention demonstrate an ICso for inhibition of positive strand HCV-RNA replication that is +30% the ICso for inhibition of negative strand HCV- RNA replication.

"Treating"or"treatment"is intended to mean at least the mitigation of a disease condition (acute, chronic, latent, etc.) in a mammal, such as a human, that is caused by an infectious RNA-containing virus. The methods of treatment for mitigation of a disease condition include the use of the compounds in this invention in any conventionally acceptable manner, for example for prevention, retardation, prophylaxis, therapy or cure of a disease. The compounds of Formula I of this invention are particularly useful for the treatment of acute, chronic or latent HCV diseases, such as acute and chronic hepatitis infection, hepatocellular carcinoma, liver fibrosis, or other HCV-related diseases. The compounds of Formula I of this invention may also be useful for treatment of diseases caused by infectious RNA-containing viruses other than HCV, including, but not limited to, Dengue, HIV or picornaviruses. Chronic fatigue syndrome is another disease that may be treatable using the compounds of this invention.

An inventive compound of Formula I, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof may be administered as a pharmaceutical composition in any pharmaceutical form recognizable to the skilled artisan as being suitable. Suitable pharmaceutical forms include solid, semisolid, liquid, or lyophilized formulations, such as tablets, powders, capsules, suppositories, suspensions, liposomes, and aerosols. Pharmaceutical compositions of the invention may also include suitable excipients, diluents, vehicles, and carriers, as well as other pharmaceutically active agents, depending upon the intended use or mode of administration. Administration of a compound of the Formula I, or a tautomer thereof, or pharmaceutically acceptable salt or solvate thereof, may be performed according to any of the generally accepted modes of administration available to those skilled in the art. The compounds of this invention may be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, topical, transdermal, or transmucosal administration. For systemic administration, oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets and liquid preparations such as syrups, elixirs and concentrated drops. Alternatively, injection (e. g. , parenteral administration) may be used, e. g. , intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably, in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. The compounds of the invention may also be formulated in liposome-containing preparations, particularly liposome-containing preparations useful for delivery of the compounds of this invention to the liver or potentially to nonhepatic reservoirs of infection. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives.

In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art. We also should consider liposome mediated delivery to liver as this technology has developed significantly in past year.

Compositions containing a compound of Formula I, or a tautomer thereof, or pharmaceutically acceptable salt or solvate thereof, which are active when given orally can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil. olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include starch, calcium sulfate dihydrate, magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and may be incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula I, or a tautomer thereof, or pharmaceutically acceptable salt or solvate thereof, which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is formulated and administered in a unit dosage form.

For oral application, for example, one or more tablets or capsules may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. A dose of the pharmaceutical composition contains at least a therapeutically effective amount of the active compound (i. e. , a compound of Formula I, or a tautomer thereof, or pharmaceutically acceptable salt or solvate thereof). The selected dose may be administered to a mammal, for example, a human patient, in need of treatment mediated by inhibition of HCV activity by any known or suitable method of administering the dose, including: topically, for example, as an ointment, or cream; orally; rectally, for example, as a suppository; parenterally by injection; or continuously by intravaginal, intranasal, intrabronchial, intraaural, or intraocular infusion.

Treatment of all forms of infection or disease (acute, chronic, latent etc) or as prophylaxis with these compounds (or their salts etc.) may be achieved using the compounds of this invention as a monotherapy, in dual or multiple combination therapy, such as in combination with other antivirals; in combination with an interferon, in combination with an interferon

and ribavirin, or in combination with one or more agents which include but are not limited to: immunomodulatory agents (such as cytokines, suppressors of cytokines and/or cytokine signalling, or immune modifiers, adjuvants and the like); immunomodulatory agents that enhance the body's immune system (such as vitamins, nutritional supplements, antioxidant compositions, vaccines or immunostimulating complexes, such as vaccines comprising a multimeric presentation of an antigen and adjuvant); other direct antiviral agents; indirect antiviral agents or agents which target viral RNA and impair translation or replication or modulate signalling or cellular host factors, or host-viral interface, immunoglobulins; antisense agents against HCV; peptide-nucleic acid conjugates; oligonucleotides; ribozymes; polynucleotides; anti-inflammatory agents; pro-inflammatory agents; antibiotics; hepatoprotectants; or any anti-infectious agents and the like; or combinations thereof.

Moreover, the additional agents may be combined with the compounds of this invention to create a single dosage form. Alternatively, these additional agents may be separately administered as part of a multiple dosage form. As used herein the term"an interferon"is intended to mean any form interferon, which includes, but is not limited to natural or recombinant forms of alpha, beta or gamma interferons, albumin-linked interferons, or pegylated interferons.

Compounds of the present invention include: 2-benzyl-l-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1,2, 4] thiadiazin-3-yl)-5- hydroxy-1, 2-dihydro-pyrazol-3-one; 1-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl) -5-hydroxy-2- (3-methylbutyl)-1, 2-dihydro-pyrazol-3-one; and 1-tert-butyl-2- (3, 3-dimethylbutyl)-4- (1, 1-dioxo-1, 4-dihydro-1- benzo [1, 2,4] thiadiazin-3-yl)-5-hydroxy-1, 2-dihydro-pyrazol-3-one; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

Preferred compounds of this invention include 1-tert-butyl-4- (1, 1-dioxo-1,4- dihydro-1-benzo [1,2, 4] thiadiazin-3-yl)-5-hydroxy-2- (3-methylbutyl)-1, 2-dihydro-pyrazol-3- one and 1-tert-butyl-2-(3, 3-dimethylbutyl)-4-(1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl)-5-hydroxy-1, 2-dihydro-pyrazol-3-one; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

Also included in the present invention are pharmaceutically acceptable salt complexes. Preferred are the ethylene diamine, sodium, potassium, calcium, ethanolamine, hydrochloride, hydrobromide, maleate and trifluoroacetate salts of the above-listed compounds.

GENERAL SYNTHETIC METHODS This invention is also directed to methods for the synthesis of the compounds of Formula I and tautomers thereof.

Included in the present invention is a process according to Scheme 1 for the synthesis of the compounds: Scheme 1 Conditions: i."R'-aldehyde", Et20, MgS04, Et3N ; ii. ClCO2Et, pyr. , EtOAc or ClCO2Et, n- BuLi, THF or ClCO2Et, i-Pr2NEt, DMAP, CH2C12 ; iii. H2, Pd/C, MeOH ; iv. DCC, CH2Cl2, DMF; v. KOt-Bu, 2-methyl-2-propanol or NaOEt, EtOH An appropriately substituted alkyl hydrazine (a) may be condensed with an appropriate aldehyde or ketone such as benzaldehyde, isovaleraldehyde, or 3,3- dimethylbutyraldehyde in the presence of an appropriate drying agent such as magnesium sulfate in an appropriate solvent such as diethyl ether to afford an alkyl hydrazone which may be carboxyalkylated with an appropriate chloroformate such as ethyl chloroformate in the presence of an appropriate base such as pyridine, n-butyl lithium, or diisopropylethylamine with N, N-dimethylaminopyridine in an appropriate solvent such as ethyl acetate, tetrahydrofuran, or methylene chloride and then reduced under hydrogenation conditions with an appropriate catalyst such as palladium and carbon in an appropriate solvent such as methanol to afford the dialkylhydrazine carboxylate (b). Compounds of Formula I are prepared by the coupling of dialkylhydrazine carboxylate (b) with an appropriate acid (c) such as (1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl) -acetic acid in the presence of an appropriate amide coupling reagent such as dicyclohexylcarbodiimide in an appropriate solvent such as dichloromethane or dimethylformamide followed by cyclization with an appropriate base such as potassium tert-butoxide or sodium ethoxide in an appropriate solvent such as 2-methyl-2-propanol or ethanol.

The activity of the inventive compounds as inhibitors of HCV activity may be measured by any of the suitable methods known to those skilled in the art, including in vivo and in vitro assays. For example, the HCV NS5B inhibitory activity of the compounds of Formula I was determined using standard assay procedures described in Behrens et al., EMBO J. 15: 12-22 (1996), Lohmann et al., Virology 249: 108-118 (1998) and Ranjith- Kumar et al. , J. Virology 75: 8615-8623 (2001). Recently, cell-based replicon systems for HCV have been developed, in which the nonstructural proteins stably replicate subgenomic viral RNA in Huh7 cells (Lohmann et al., Science (1999) and Blight et al. , Science (2000).

In the absence of a purified, functional HCV replicase consisting of viral non-structural and host proteins, our understanding of Flaviviridae RNA synthesis comes from studies using active recombinant RdRps and validation of these studies in the HCV replicon system.

Inhibition of recombinant purified HCV polymerase with compounds in in vitro biochemical assays may be validated using the replicon system whereby the polymerase exists within a replicase complex, associated with other viral and cellular polypeptides in appropriate stoichiometry. Demonstration of cell-based inhibition of HCV replication may be more predictive of in vivo function than demonstration of HCV NS5B inhibitory activity in in vitro biochemical assays.

Advantageously, the compounds of this invention inhibit both positive and negative strand HCV-RNA replication. The following methods have been developed and used for determining the positive and negative strand HCV-RNA replication inhibition activity of the compounds of this invention.

Test Method 1 Method for positive strand replicon HCV-RNA detection in replicon cells Replicon cells were plated at 3 X 103 cells per well in a 96-well plate plates at 37 °C and 5% COz in DMEM containing 10% FCS, 1% NEAA and 1 mg/ml Geneticin. After allowing 4 h for cell attachment, 1 1Il of compound dilution was added to the medium (n = 8 wells per dilution). Briefly, eleven 2.5-fold dilutions of 1 mM stock test compound in DMSO were prepared with final concentration ranging from 10000 nM to 1.0 nM. Plates were incubated for 40 h, until reaching 80% confluence. After removal of medium, 150 gel Buffer RLT (Qiagen, Valencia, California, US) was added to each well and RNA purified according to manufacturer's recommendations (Qiagen RNAeasy) and were eluted twice in 45 ul dH20 prior to RT-PCR.

Approximately 40 jll ofTaqMan EZ RT-PCR (Applied Biosystems, Foster City, California, US) master mix (1X TaqMan EZ Buffer, 3 mM Mn (OAc) 2,0. 3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP, 0.6 mM dUTP, 0.2 mM neo-forward, 0.2 mM neo-reverse, 0.1 mM neo-probe, 1X Cyclophilin Mix, 0.1 Unitl, ul rEtll DNA Polymerase, 0. 01 Unit/ul AmpErase UNG, and H20 to 40 p. l) was added to each tube of 96-tube optical plate along with 10 pi of RNA elution. Primers and probes specific for the positive strand RNA detection of neomycin gene were: neo-forward: 5'CCGGCTACCTGCCCATTC3' (SEQ ID NO 1); neo-reverse: 5'CCAGATCATCCTGATCGACAAG3' (SEQ ID NO 2); neo-probe: 5FAM-ACATCGCATCGAGCGAGCACGTAC-TAMRA3' (SEQ ID NO 3). For negative strand RNA detection, the cDNA primer used was 5'ACA TGC GCG GCA TCT AGA CCG GCT ACC TGC CCA TTC3' (SEQ ID NO 4); neo-forward: 5'ACA TGC GCG GCA TCT AGA3' (SEQ ID NO 5); 5'CCAGATCATCCTGATCGACAAG3' (SEQ ID NO 6); neo reverse: 5'CCG GCT ACC TGC CCA TTC3' (SEQ ID NO 6); neo probe: 5FAM-ACA TCG CAT CGA GCG AGC ACG TAC-TAMRA3' (SEQ ID NO 7). Additionally, the PDAR control reagent human cyclophilin was used for normalization. Samples were mixed briefly and placed in an ABI7700 (Applied Biosystems) at 50°C, 2 min; 60°C, 30 min; and 95°C, 5 min, with cycling parameters set to 94°C, 20 s; 55°C, 1 min for 40 cycles. The relative cDNA levels for neo and cyclophilin were determined compared to DMSO-only treated controls and the ratio of neo : cyclophilin was used for ICSO calculation (n = 8).

Test Method 2 Method for negative strand replicon HCV-RNA detection in replicon cells To achieve strand-specific detection, a primer containing HCV RNA sequences and an 18 base tag of nonrelated sequence at the 5'end was for the reverse transcription (RT) reaction, 5'ACATGCGCGGCATCTAGACCGGCTACCTGCCCATTC3' (SEQ ID NO 8). A Thermoscript-RT-PCR system (Invitrogen) was used for the RT reaction according to the manufacturer's protocol, with approximately 9 pi of the cell-harvested RNA and 1 ul of primer (10 uM) incubated with RT at 60°C for 1 h. Following that incubation, 2 ptl of cDNA product containing the 5'tag was amplified for TaqMan quantification using the 48 pi of TaqMan Universal Master Mix (Applied Biosystems) as well as primers, neo-tag: 5'ACA TGC GCG GCA TCT AGA3' (SEQ ID NO 5); neo reverse: 5'CCAGATCATCCTGATCGACAAG3' (SEQ ID NO 6); and neo probe: 5'FAM-ACA TCG CAT CGA GCG AGC ACG TAC-TAMRA3' (SEQ ID NO 7). Samples were mixed briefly and placed in an ABI7700 (Applied Biosystems) at 50°C, 2 min ; 95°C, 10 min, with cycling parameters set to 94°C, 15 s; 55°C, 1 min for 40 cycles. The negative strand copy number in each reaction was determined using linear regression analysis based on the slope and intercept generated with a negative strand copy standard curve. The negative strand copies per cell were determined by dividing the total negative strand copies per reaction by the total cells per reaction.

Through routine experimentation, including appropriate manipulation and protection of any chemical functionality, synthesis of the compounds of Formula I is accomplished by methods analogous to those above and to those described in the following Experimental section.

In the following Examples, proton NMR spectra were obtained using Bruker ARX- 300 or Avance-400 NMR spectrometers.

Liquid Chromatography-Mass Spectroscopy analysis was conducted using a Sciex API 150EX instrument [1 x 40 mm Aquasil (C18) column, gradient 4.5%-90% acetonitrile-water (0.02% TFA) over 3.2 min, detection by mass, UV at 214 nM and by evaporative light-scattering].

Example 1 2-benzyl-1-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl) -5-hydroxy- 1,2-dihydro-pyrazol-3-one a) N-benzylidene-N'-tert-butylhydrazine A solution of tert-butylhydrazine hydrochloride (1.00 g, 8.02 mmol) in anhydrous diethyl ether (10.0 mL) was treated with triethylamine (1.12 mL, 8.02 mmol), benzaldehyde (0.815 mL, 8.02 mmol), and magnesium sulfate (1.06 g, 8.82 mmol). After stirring 18 h at ambient temperature, the reaction mixture was filtered and concentrated in vacuo to give the title compound as a light yellow oil (1.40 g, 99%). MS (ES+) m/e 177 [M+H] +. b) Ethyl N'-benzylidene-N-tert-butylhydrazinecarboxylate A solution of the compound from example I (a) (0.444 g, 2.52 mmol) in anhydrous ethyl acetate (5.0 mL) was treated with pyridine (0.204 mL, 2.52 mmol) and ethylchloroformate (0.240 mL, 2.52 mmol). After stirring 18 h at ambient temperature, the reaction mixture was concentrated in vacuo and taken on without purification. MS (ES+) m/e 249 [M+H] +. c) Ethyl N'benzyl-N-tert-butylhydrazinecarboxylate A solution of the compound from Example I (b) (approx. 0.625 g, 2.52 mmol) in methanol (10.0 mL) with 10 % palladium on charcoal (0.268 g, 0.252 mmol) was stirred under an atmosphere of hydrogen for 3 h. The mixture was filtered through CeliteS), which was then washed with methanol. The resulting filtrate was concentrated in vacuo and purified via flash column chromatography (0-30% ethyl acetate in hexanes) to give the title compound as a clear, colorless oil (0.125 g, 20%, 2 steps). 1H NMR (400MHz, CDCI3) 8 7. 38-7. 25 (m, 5H), 4.20 (q, J = 7 Hz, 2H), 4.14 (s, 2H), 4.14-3. 70 (br s, 1H), 1.41 (s, 9H), 1.33 (t, J= 7 Hz, 3H). MS (ES+) m/e 251 [M+H] +. d) 1,1-dioxo-1, 4-dihydro-1-benzo [1,2, 4] thiadiazin-3-yl-acetic acid A solution of ethyl 1, l-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl-acetate (prepared by the method of Kovalenko, S. N.; Chernykh, V. P.; Shkarlat, A. E.; Ukrainets, I.

V.; Gridasov, V. I. ; Rudnev, S. A. Che) n. Heterocycl. Contpd. (Engl. Trans.) 1998, 34, 791) (10.2 g, 38.0 mmol) in tetrahydrofuran (50.0 mL) was treated with 6M aqueous hydrochloric acid (100.0 mL). After stirring 1 wk at ambient temperature, the reaction mixture was diluted with brine, extracted thrice with ethyl acetate, dried over magnesium sulfate, filtered, and concentrated in vacuo to give the title compound as a white solid (8.30 g, 91%). 1H NMR (400MHz, D6-DMSO) 5 13.0 (br s, 1H), 12.9 (s, 1H), 7.80 (dd, J = 1, 8 Hz, 1H), 7.68 (dt, J= 1,7 Hz, 1H), 7.45 (dt, J = 1, 7 Hz, 1H), 7.31 (d, J= 8 Hz, 1H), 3.57 (s, 2H). MS (ES+) m/e 241 [M+H] +. e) Ethyl N'-benzyl-N-tert-butyl-N'-[2-(1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl) - ethanoyl]-hydrazinecarboxylate A solution of the compound from example 1 (c) (0.123 g, 0.491 mmol) and the compound from example 1 (d) (0.118 g, 0.491 mmol) in anhydrous methylene chloride (3.0 mL) and anhydrous dimethylformamide (0.5 mL) was treated with dicyclohexylcarbodiimide (1M solution in methylene chloride, 0.491 mL, 0.491 mmol).

After stirring 2 h at ambient temperature, the reaction mixture was filtered, concentrated in vacuo, and taken on without purification. MS (ES+) m/e 473 [M+H] +. f) 2-benzyl-1-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1,2, 4] thiadiazin-3-yl)-5-hydroxy- 1,2-dihydro-pyrazol-3-one A solution of the compound from example I (e) (approx. 0.232 g, 0.491 mmol) in anhydrous 2-methyl-2-propanol (5.0 mL) was treated with potassium tert-butoxide (0.138 g, 1.23 mmol). After stirring 24 h at ambient temperature, additional potassium tert-butoxide (0. 138 g, 1.23 mmol) was added, the solution was stirred for another 24 h at ambient temperature, and a third portion of tert-butoxide (0. 138 g, 1.23 mmol). After stirring another 24h at ambient temperature, the reaction mixture was quenched with 1M aqueous hydrochloric acid, diluted with brine, extracted twice with methylene chloride, dried over magnesium sulfate, filtered, concentrated in vacuo, and purified via flash column chromatography (30-80% ethyl acetate in hexanes) to give the title compound as an off- white solid (0.035 g, 17%, 2 steps). 1H NMR (400MHz, D6-DMSO) 8 12.5 (s, 1H), 7.62 (dd, J = 2,8 Hz, 1H), 7.50 (dt, J = 2,8 Hz, 1H), 7.27-7. 22 (m, 2H), 7.25 (dt, J = 2,8 Hz, 1H), 7.21-7. 16 (m, 3H), 7.17 (dd, J = 2, 8 Hz, 1H), 4.58 (s, 2H), 1.30 (s, 9H). MS (ES+) m/e 427 [M+H] +.

Example 2 l-tert-butyl-4-(1, 1-dioxo-1, 4-dihydro-1-benzo [1, 2,4] thiadiazin-3-yl)-5-hydroxy-2- (3- methylbutyl)-1, 2-dihydro-pyrazol-3-one Following the procedures of examples 1 (a), 1 (b), 1 (c), 1 (d), and 1 (f), except substituting isovaleraldehyde for benzaldehyde, diisopropylethylamine with N, N- dimethylaminopyridine in methylene chloride for pyridine in ethyl acetate, and sodium ethoxide in ethanol at reflux for potassium tert-butoxide in 2-methyl-2-propanol at ambient temperature, the title compound was obtained as a light brown solid. 1H NMR (400MHz, D6-DMSO) 8 12.5 (s, 1H), 7.64 (d, J = 7 Hz, 1H), 7.52 (t, J = 8 Hz, 1H), 7.25 (t, J = 7 Hz, 1H), 7.21 (d, J = 8 Hz, 1H), 3.44 (m, 2H), 1.45-1. 35 (m, 1H), 1.33 (s, 9H), 1.33-1. 22 (m, 2H), 0.83 (d, J = 6 Hz, 6H). MS (ES+) m/e 407 [M+H] +.

Example 3 1-tert-butyl-2- (3, 3-dimethylbutyl)-4- (1, 1-dioxo-1, 4-dihydro-l-benzo [1, 2,4] thiadiazin-3-yl) - 5-hydroxy-1,2-dihydro-pyrazol-3-one Following the procedure of example 2, except substituting or 3,3- dimethylbutyraldehyde for

isovaleraldehyde, the title compound was obtained as a light brown solid. 1H NMR (400MHz, D6-DMSO) 8 12.5 (s, 1H), 7.64 (d, J = 8 Hz, 1H), 7.52 (dt, J = 1,7 Hz, 1H), 7.25 (t, J = 8 Hz, 1H), 7.21 (d, J = 8 Hz, 1H), 3.44 (m, 2H), 1.33 (s, 9H), 1.29-1. 23 (m, 2H), 0.85 (s, 9H). MS (ES+) m/e 421 [M+H] +.

All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses how to make and use the present invention.

However, this invention is not limited to the particular embodiments described hereinabove, but includes all modification thereof within the scope of the appended claims and their equivalents. Those skilled in the art will recognise through routine experimentation that various changes and modifications can be made without departing from the scope of this invention.

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Note: OCR Text

What is claimed is: 1. A compound of Formula I : wherein: R1 and R2 are each independently selected from the group consisting of: Cl-Cl0 alkyl, C2-Clo alkenyl, C2-Clo alkynyl, C3-C8 cycloalkyl, aryl or heteroaryl, where said alkyl, alkenyl, alkynyl, cycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from Cl-C6 alkyl, C3-C6 cycloalkyl, heterocycloalkyl, aryl, heteroaryl, halogen, nitro, cyano,-C (O) OR7, -C (O) R',-OR',-SR', -S(O)R7, -S(O)2R7, -SO2NR8R9, -CONR8R9, -N(R8) C (O) R7, -N (R8) C (O) OR7, -OC(O)NR8R9, -N(R8) C (O) NR8R9,-P (O) (OR7)2, -SO2NR8R9, -N(R8)SO2R7, and -NR8R9; wherein said cycloalkyl, heterocycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from -NR8R9, Cl-C6 alkyl, C1-C6 haloalkyl, halogen, cyano and nitro; R3 is hydrogen, halogen, cyano, Cl-C6 alkyl,-OH, or-OCl-C4 alkyl ; R4 is hydrogen, halogen, cyano, Cl-C6 alkyl, -OH, -OC1-4 alkyl, Cl-C4 haloalkyl, nitro or amino; R5 is H, nitro, cyano, halogen, -C (O) OR,-C (O) R7,-OR7,-SR7,-S (O) R7,-S (O) 2R7, -NR8R9, protected -OH, -CONR8R9, -N(R8) C (O) R7, -N (R8) C (O) OR7,-OC (O) NR8R9, -N(R8) C (O) NR8R9,-P (O) (OR7) 2, -SO2NR8R9, -N (R8) SO2R7, Cl-Cl0 alkyl, C2-C10 alkenyl, C2-C10 alkynyl, C3-C6 cycloalkyl, aryl or heteroaryl, where said alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is unsubstituted or substituted with one or more substituents independently selected from Cl-C6 alkyl, Cl-C6 haloalkyl, aryl, heteroaryl, halogen,-OR7, -SR7, -NR8R9, cyano and nitro; R6 is H, halogen, Cl-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, -OR7, -SR7, -NR8R9, cyano or nitro; wherein each R7 is independently selected from the group consisting of H, Cl-Clo alkyl, C2-C10 alkenyl, C2-C10 alkynyl, C3-C8 cycloalkyl, heterocycloalkyl, C6-C12 aryl, heteroaryl, or C6-Cl2 aryl-Cl C6 alkyl-, where said alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl or arylalkyl group is unsubstituted or substituted with one or more substituents independently selected from C1-C4 alkyl, haloalkyl, halogen, hydroxyl,-O (C1-C4 alkyl), cyano, nitro,-N (C1-C4 alkyl)(C1-C4 alkyl), -NH(C1-C4 alkyl), -NH2, aryl, heteroaryl,-C02 (Cl-C4 alkyl), -CO2H, -CON(C1-C4 alkyl) (C1-C4 alkyl),-CONH (Cl-C4 alkyl),-CONH2, -N(C1-C4 alkyl) CON (Cl-C4 alkyl) (C-C4 alkyl),-NHCON (Cl-C4 alkyl) (Cl-C4 alkyl), -NHCONH(C1-C4 alkyl),-OCON(C1-C4 alkyl) (C1-C4 alkyl),-OCONH (Cl-C4 alkyl), OCONH2,-SO2N(C1-C4 alkyl) (Cl-C4 alkyl),-SO2NH (Cl-C4 alkyl),-CO (Cl-C4 alkyl), - CO (aryl) and-CO (heteroaryl); and R8 and R9 are each independently selected from H, C1-C10 alkyl, C3-C8 cycloalkyl, heterocycloalkyl, C6-Cn aryl, heteroaryl, C3-C8 cycloalkyl-Cl-C6 alkyl-, heterocycloalkyl-Cl-C6 alkyl-, C6-C12 aryl-C1-C6 alkyl- or heteroaryl-C1-C6 alkyl-, or R8 and R9 taken together with the nitrogen to which they are attached represent a 5-or 6-membered saturated ring optionally containing one other heteroatom selected from oxygen, sulfur, and nitrogen, where said alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkylalkyl, arylalkyl, heteroarylalkyl or 5-or 6-membered ring is unsubstituted or substituted with one or more substituents independently selected from Cl-C4 alkyl, Cl-C4 haloalkyl, halogen, hydroxyl,-O (Cl-C4 alkyl), cyano, nitro, -N(C1-C4 alkyl) (Ci-C4 alkyl),-NH (C-C4 alkyl),-NH2, aryl, heteroaryl, -CO2(C1-C4 alkyl), -CO2H, -CON(C1-C4 alkyl)(C1-C4 alkyl), -CONH(C1-C4 alkyl), -CONH2, -N(C1-C4 alkyl) CON (C1-C4 alkyl) (Cl-C4 alkyl),-NHCON (Cl-C4 alkyl) (Ci-C4 alkyl), -NHCONH(C1-C4 alkyl),-OCON(C1-C4 alkyl) (C1-C4 alkyl), OCONH(C1-C4 alkyl),-SO2N(C1-C4 alkyl)(C1-C4 alkyl), -SO2NH(C1-C4 alkyl), -CO(C1-C4 alkyl), -CO (aryl) and-CO (heteroaryl); or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to claim 1, wherein: R'is C3-C8 alkyl, C3-C6 cycloalkyl-Cl-C4 alkyl-, C6 aryl-Cl-C4 alkyl-, monocyclic heterocycloalkyl-C1-C4 alkyl-or monocyclic heteroaryl-Cl-C4 alkyl ; R2 is Cl-C6 alkyl, C3-C6 cycloalkyl, monocyclic heterocycloalkyl, phenyl, monocyclic heteroaryl, C3-C6 cycloalkyl-Cl-C4 alkyl-, C6 aryl-Ci-C4 alkyl-, monocyclic heterocycloalkyl-Cl-C4 alkyl-or monocyclic heteroaryl-Cl-C4 alkyl- ; R3, R4 and R6 are each H; and Rs is H, Cl-C4 alkyl,-OH or-O (C1-C4 alkyl), wherein said Ci-C4 alkyl or -O(C1-C4 alkyl) is unsubstituted or substituted by-NH2,-CN,-CONH2, -CON (C-C4 alkyl) (C-C4 alkyl),-CONH (Ra), aryl or heteroaryl, wherein the aryl or heteroaryl is optionally unsubstituted or substituted by one or more substituents selected from the group consisting of cyano, halogen, -C (O) O (C,-C6 alkyl),-C (O) Cl-C6 alkyl,-ORa, -NRaRa,-CONRaRa, and-OCONRaRa, wherein each Ra is independently H or Cl-C4 alkyl ; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

3. A compound according to claim 1, wherein: R'is C3-C6 alkyl or benzyl; R2 is C3-C6 alkyl ; R3, R4, R5 and R6 are each H; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

4. A compound selected from the group consisting of: 2-benzyl-1-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1,2, 4] thiadiazin-3-yl)-5- hydroxy-1, 2-dihydro-pyrazol-3-one; 1-tert-butyl-4- (1, 1-dioxo-1, 4-dihydro-1-benzo [1,2, 4] thiadiazin-3-yl)-5-hydroxy-2- (3-methylbutyl) -1,2-dihydro-pyrazol-3-one ; and 1-tert-butyl-2- (3, 3-dimethylbutyl)-4- (1, 1-dioxo-1, 4-

dihydro-l- benzo [1, 2,4] thiadiazin-3-yl)-5-hydroxy-1, 2-dihydro-pyrazol-3-one; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

5. A pharmaceutically acceptable salt of the compound according to any one of claim 1-4, or tautomer thereof, wherein said pharmaceutically acceptable salt is a sodium salt or a potassium salt.

6. A method of inhibiting an RNA-containing virus which comprises contacting said virus with an effective amount of the compound according to any one of claims 1 to 5.

7. A method of treating infection caused by an RNA-containing virus which comprises administering to a subject in need thereof an effective amount of the compound according to any one of claims 1 to 5.

8. A method according to claim 7 comprising treating an HCV infection.

9. A method according to claim 6 or claim 7 comprising inhibiting hepatitis C virus.

10. A method according to claim 7, wherein said HCV infection is acute hepatitis infection, chronic hepatitis infection, hepatocellular carcinoma or liver fibrosis.

11. A method according to claim 7 comprising treating an infection caused by Dengue, HIV or a picornavirus.

12. A method according to claim 7 comprising administering said compound in combination with one or more agents selected from the group consisting of an immunomodulatory agent and an antiviral agent.

13. A method according to claim 12, wherein the immunomodulatory agent is selected from the group consisting of alpha interferon, beta interferon, gamma interferon, a cytokine, a vitamin, a nutritional supplement, an antioxidant compound, a vaccine and a vaccine comprising an antigen and an adjuvant.

14. A method according to claim 7 comprising administering said compound in combination with an interferon.

15. A method according to claim 7 comprising administering said compound in combination with an interferon and ribavirin.

16. A method according to claim 7 comprising administering said compound in combination with an interferon and levovirin.

17. A method according to claim 7 comprising administering said compound in combination with an HCV antisense agent.

18. A method according to claim 7 comprising administering said compound in combination with an immunoglobulin, a peptide-nucleic acid conjugate, an oligonucleotide, a ribozyme, a polynucleotide, an anti-inflammatory agent, a pro-inflammatory agent, an antibiotic or a hepatoprotectant.

19. A method for inhibiting replication of hepatitis C virus comprising inhibiting replication of both positive and negative strand HCV-RNA, said method comprising contacting a cell infected with said virus with an effective amount of the compound according to any one of claims 1 to 5.

20. A method of treating infection caused by hepatitis C virus comprising inhibiting replication of both positive and negative strand HCV-RNA, said method comprising administering to a subject in need thereof an effective amount of the compound according to any one of claims 1 to 5.

21. The method according to claim 19, wherein said compound substantially equally inhibits positive strand HCV-RNA

replication and negative strand HCV-RNA replication.

22. The method according to claim 20, wherein said compound substantially equally inhibits positive strand HCV-RNA replication and negative strand HCV-RNA replication.

23. Use of a compound according to any one of claims 1-5 for the preparation of a medicament for treatment of infection caused by an RNA-containing virus.

24. A method for preparing the compound of Formula I according to claim 1,, comprising the steps of : a) condensing a compound having the formula: with an aldehyde followed by carboxy alkylation and hydrogenation to form a dialkylhydrazine carboxylate having the formula: b) treating the dialkylhydrazine carboxylate formed in step a) with an acid having the formula: in the presence of an amide coupling reagent followed by treatment with a base to provide the compound of Formula I.

25. The method according to claim 24 further comprising the step of treating the compound of Formula I with a base to provide the pharmaceutically acceptable salt of the compound of Formula I.